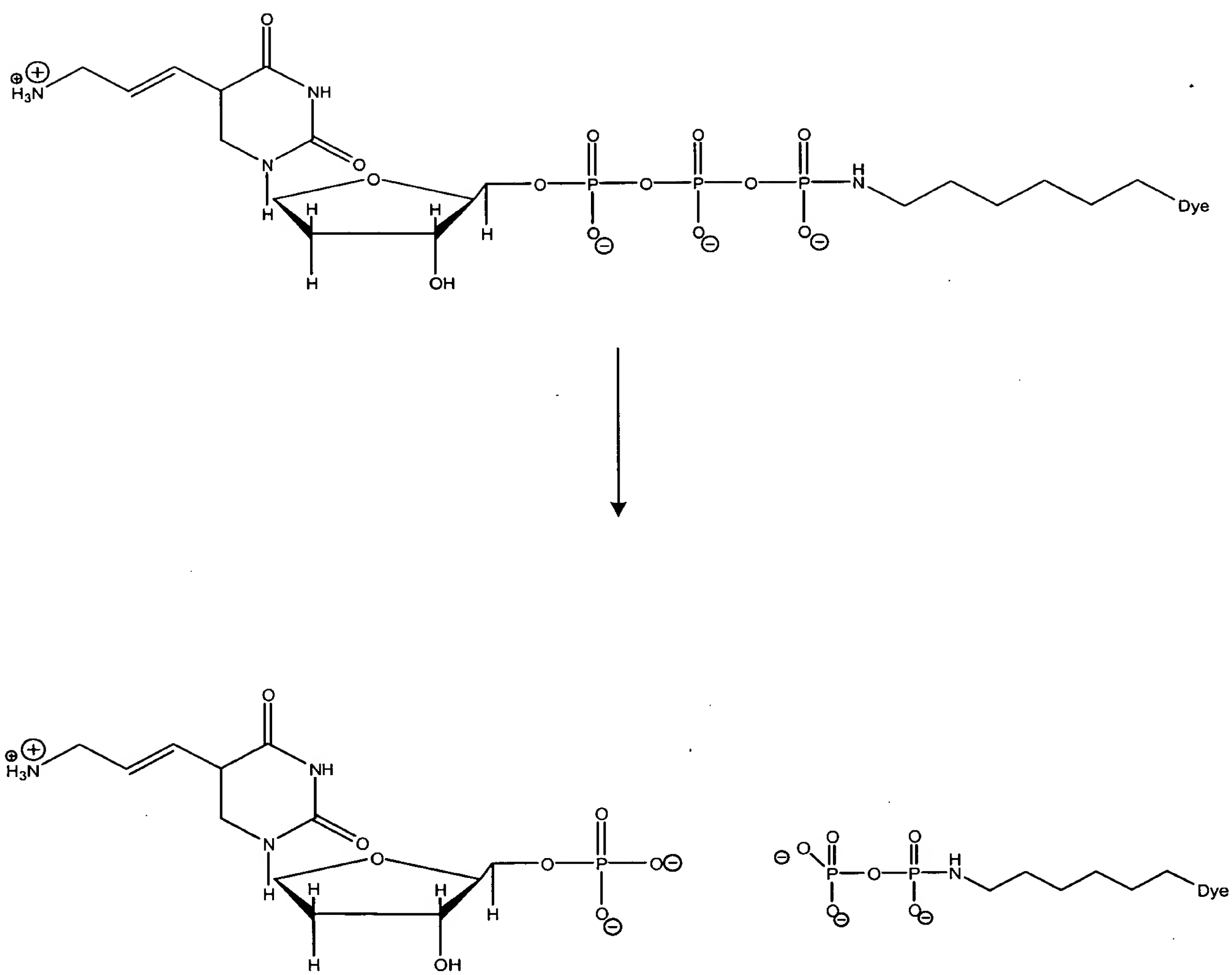


FIG. 1

	IDEAL CONDITION: ALL BASE AND F ADDUCTS FULLY CHARGED					IN PURE WATER
	Charge on indicated moiety		Net charge			pH 7.0
NO.	NUCLEOBASE	F	NP PROBE	PPi-F	Change	Change
1	-3	-3	-9	-6	3	3.25
2	-3	-2	-8	-5	3	3.25
3	-3	-1	-7	-4	3	3.25
4	-3	0	-6	-3	3	3.25
5	-3	1	-5	-2	3	3.25
6	-3	2	-4	-1	3	3.25
7	-3	3	-3	0	3	3.25
8	-2	-3	-8	-6	2	2.26
9	-2	-2	-7	-5	2	2.26
10	-2	-1	-6	-4	2	2.26
11	-2	0	-5	-3	2	2.26
12	-2	1	-4	-2	2	2.26
13	-2	2	-3	-1	2	2.26
14	-2	3	-2	0	2	2.26
15	-1	-3	-7	-6	1	1.26
16	-1	-2	-6	-5	1	1.26
17	-1	-1	-5	-4	1	1.26
18	-1	0	-4	-3	1	1.26
19	-1	1	-3	-2	1	1.26
20	-1	2	-2	-1	1	1.26
21	-1	3	-1	0	1	1.26
22	0	-3	-6	-6	0	0.26
23	0	-2	-5	-5	0	0.26
24	0	-1	-4	-4	0	0.26
25	0	0	-3	-3	0	0.26
26	0	1	-2	-2	0	0.26
27	0	2	-1	-1	0	0.26
28	0	3	0	0	0	0.26
29	1	-3	-5	-6	-1	-0.74
30	1	-2	-4	-5	-1	-0.74
31	1	-1	-3	-4	-1	-0.74
32	1	0	-2	-3	-1	-0.74
33	1	1	-1	-2	-1	-0.74
34	1	2	0	-1	-1	-0.74
35	1	3	1	0	-1	-0.74
36	2	-3	-4	-6	-2	-1.74
37	2	-2	-3	-5	-2	-1.74
38	2	-1	-2	-4	-2	-1.74
39	2	0	-1	-3	-2	-1.74
40	2	1	0	-2	-2	-1.74
41	2	2	1	-1	-2	-1.74
42	2	3	2	0	-2	-1.74
43	3	-3	-3	-6	-3	-2.74
44	3	-2	-2	-5	-3	-2.74
45	3	-1	-1	-4	-3	-2.74
46	3	0	0	-3	-3	-2.74
47	3	1	1	-2	-3	-2.74
48	3	2	2	-1	-3	-2.74
49	3	3	3	0	-3	-2.74

FIG. 2

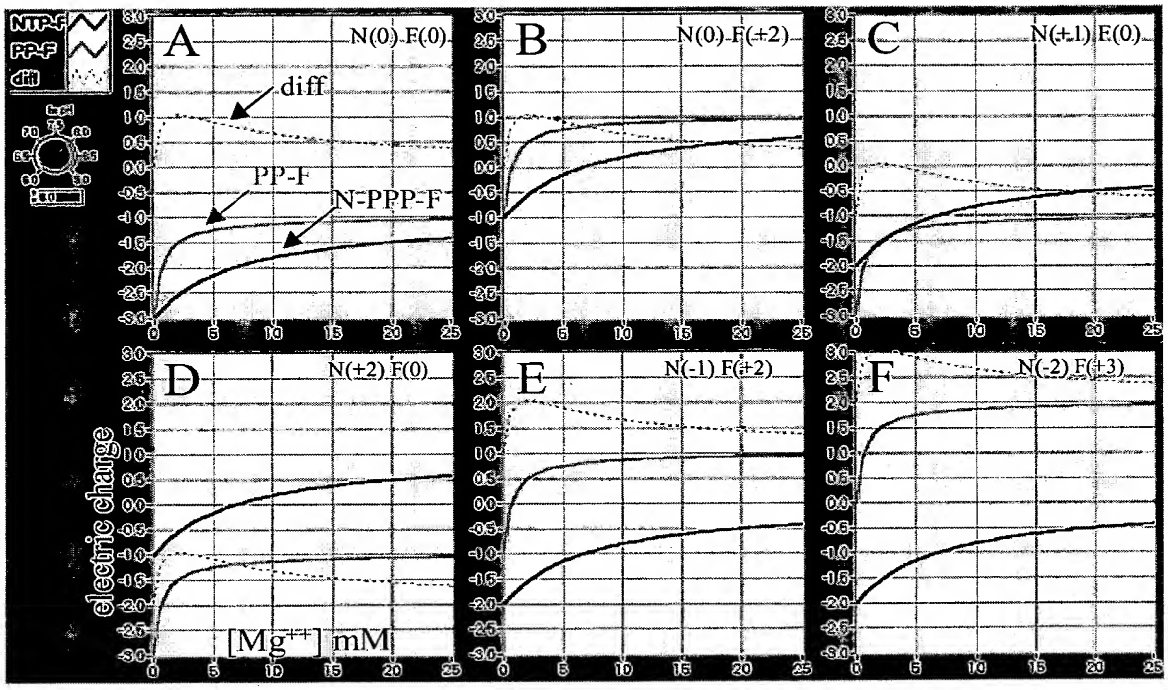
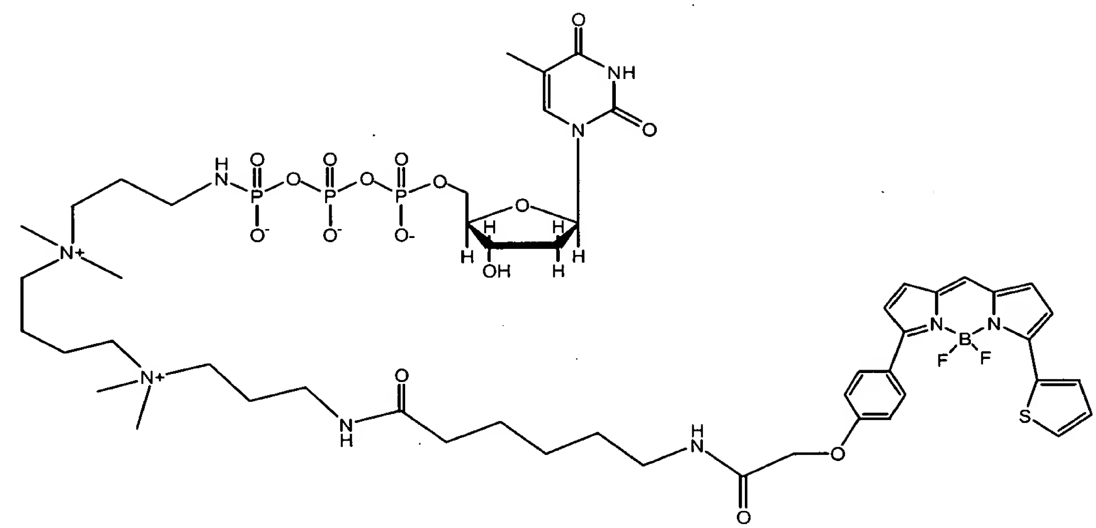

NTP-F
PP-F
diff
A
N(0) F(0)
diff
PP-F
N-PPP-F
B
N(0) F(+2)
C
N(+1) F(0)
D
N(+2) F(0)
E
N(-1) F(+2)
F
N(-2) F(+3)
electric charge
[Mg++] mM

FIG. 3



dTTP-BQS-BTR

FIG. 4

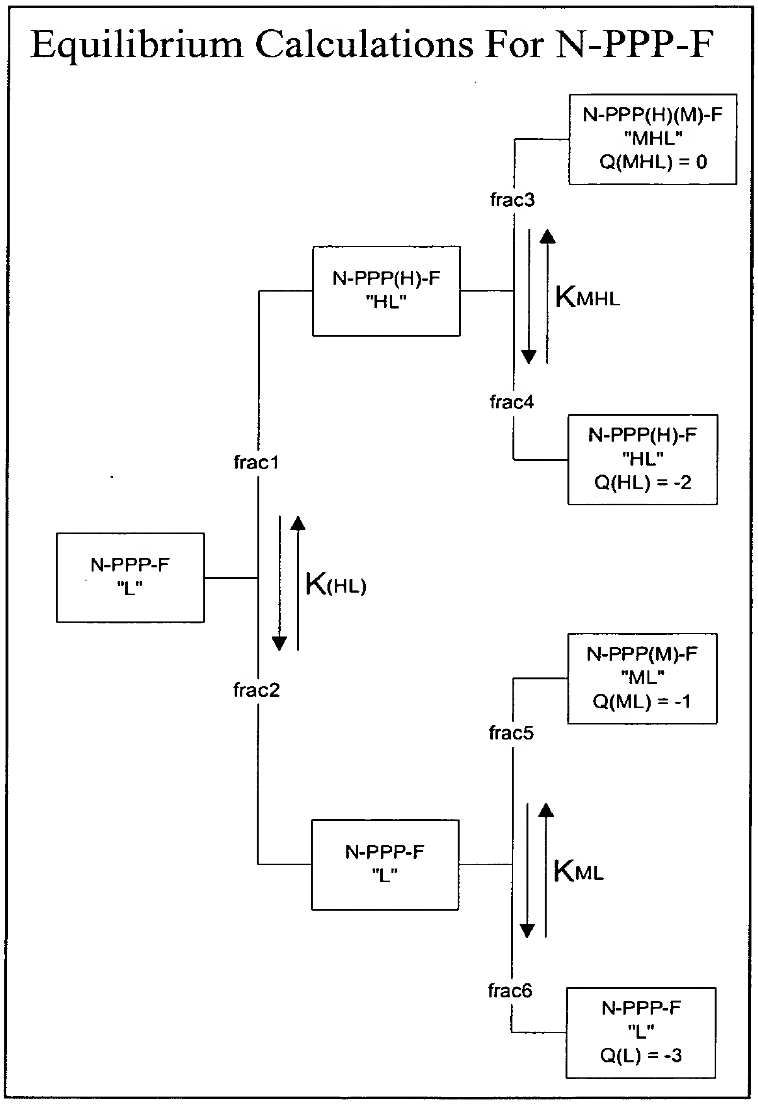

Equilibrium Calculations For N-PPP-F
N-PPP-F
"L"
K(HL)
frac1
frac2
N-PPP(H)-F
"HL"
N-PPP-F
"L"
KMHL
frac3
frac4
KML
frac5
frac6
N-PPP(H)(M)-F
"MHL"
Q(MHL) = 0
N-PPP(H)-F
"HL"
Q(HL) = -2
N-PPP(M)-F
"ML"
Q(ML) = -1
N-PPP-F
"L"
Q(L) = -3

FIG. 5

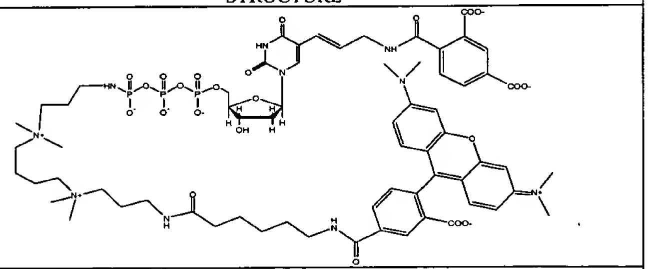
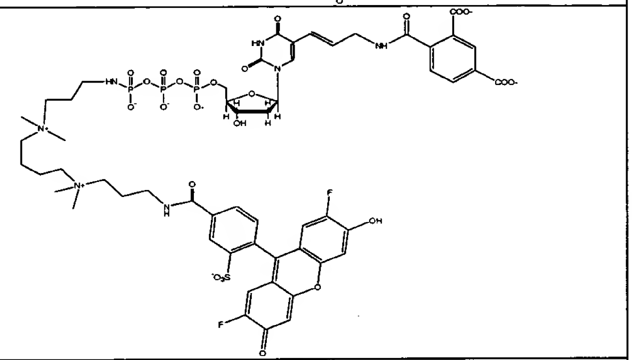
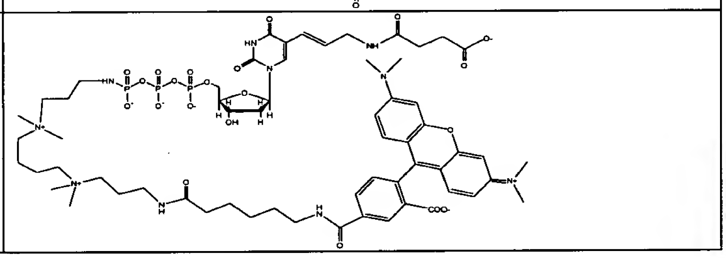
COMPOUND	CHARGE	NAME	STRUCTURE
50	N = -2 F = +2	DBA-U-BQS-TAMRA X	
51	N = -2 F = +1	DBA-U-BQS-Oregon 500	
52	N = -1 F = +2	SUC-U-BQS-TAMRA X	

FIG. 6A

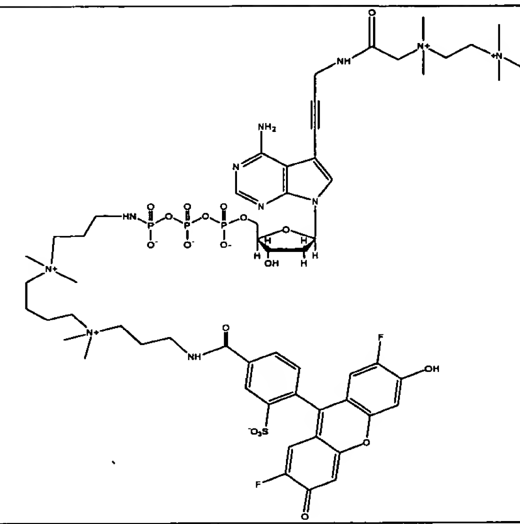
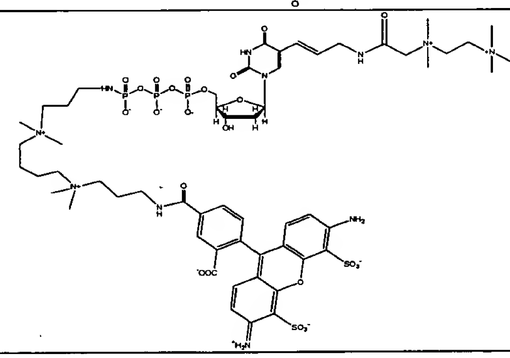
53	N = +2 F = +1	PAA-A-BQS-Oregon 500	
54	N = +2 F = 0	PAA-U-BQS-Alexa 488	

FIG. 6B

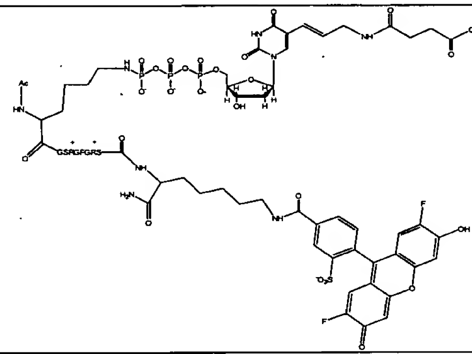
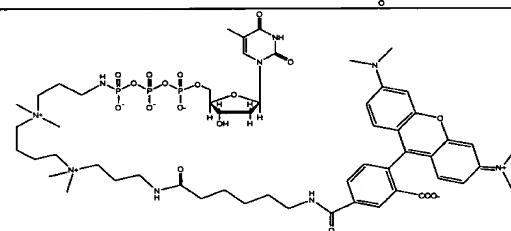
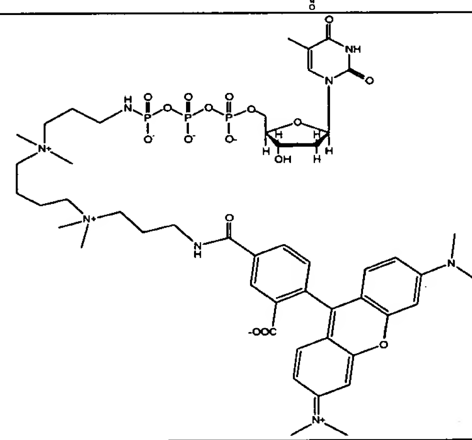
55	N = -1 F = +1	Suc-U-peptide+2-Oregon 500	
56	N = 0 F = 2	T-BQS-TAMRA X	
57	N = 0 F = 2	T-BQS-TAMRA	

FIG. 6C

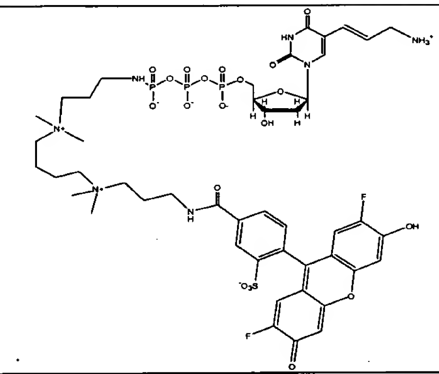
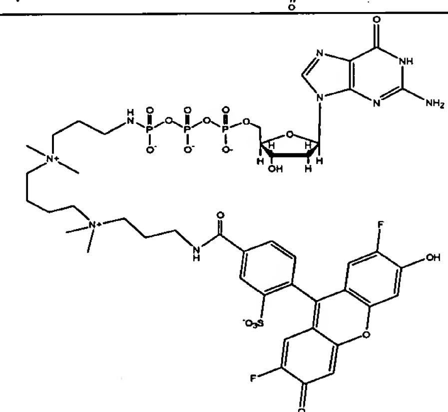
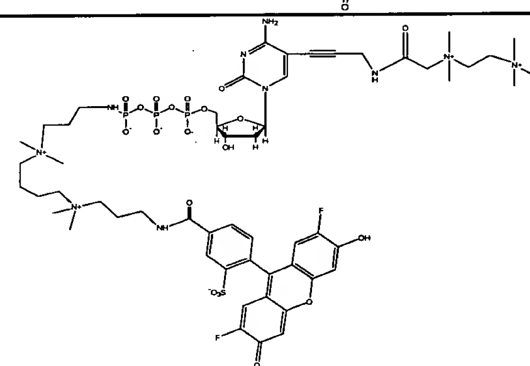
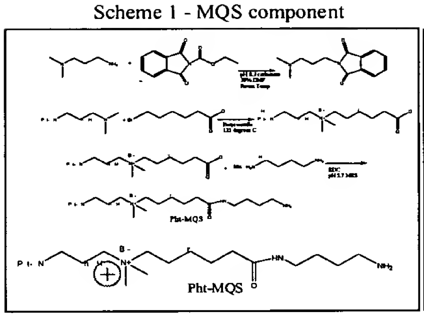
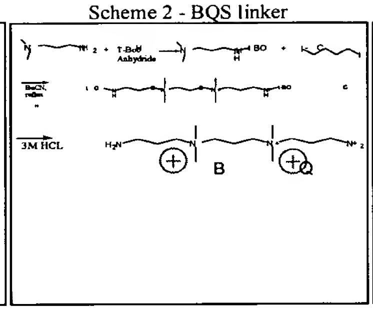
58	N = +1 F = +1	AA-U-BQS-Oregon 500	
59	N = 0 F = +1	G-BQS-Oregon 500	
60	N = +2 F = +1	PAA-C-BQS-Oregon 500	

FIG. 6D

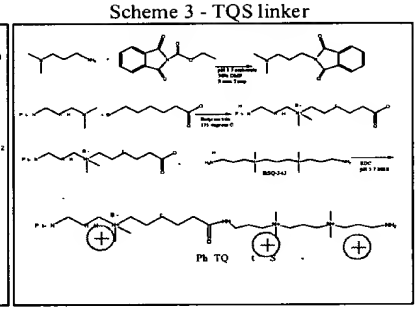
Scheme 1 - MQS component



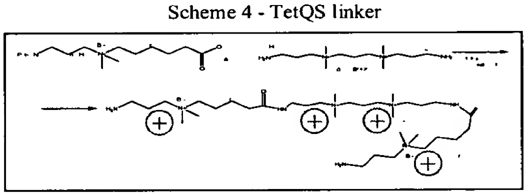
Scheme 2 - BQS linker



Scheme 3 - TQS linker

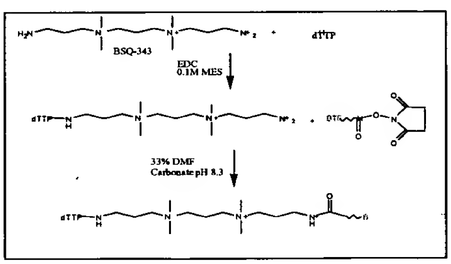


Scheme 4 - TetQS linker



Scheme 5- Protect AA-dUTP

Use same chemistry as in Scheme 1, except the amine is the aminoallyl group of AA-dUTP. shown that deprotection can be accomplished in 1M NaOH, room temp, 2 hours, without degrading triphosphates.



Scheme 6 - Coupling nucleotide, linker and dye (BTR is BodipyTR dye shown as the succinimide ester). We use this chemistry routinely to make γ-dNTPs (e.g, see cpd of Fig 13A)).

FIG. 6E

(925) 472-5000
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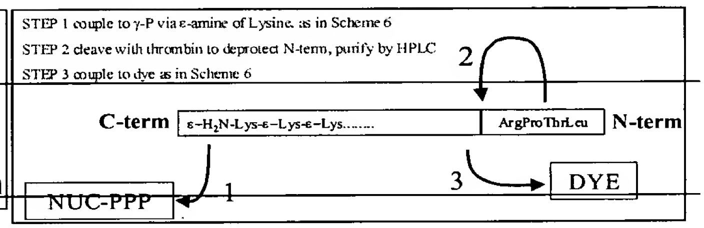
Nucleic Acid Sequencing Using Charge-Switch Nucleotides
John G.K. Williams et al.
Filed Herewith
020031-000820US
Reg. No. 39,381
Appl. No. Unassigned

Scheme 7 - Peptide linkers (shown in C-to-N direction)

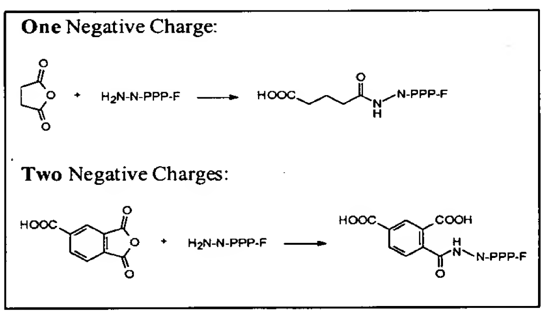
Pep(+2) $CONH_2$-KKKRYGTSRSYGS**RPTL**-N-acetyl (ε-NH_2 on K)

Pep(+3) $CONH_2$-KKKRYGTSRSYRS**RPTL**-N-acetyl (ε-NH_2 on K)

Scheme 8 - Peptide Deprotection By Thrombin Cleavage



Scheme 9 - Add carboxylate to aminoally-dUTP



Scheme 10 - γ-dNTP With Carboxylated Base

1. NH2-dU-PPP + **Pht** (of Scheme 1) ⟶
2. **Pht**-NH-dU-PPP + εNH2-KKK-pep-**RPTL** ⟶
3. **Pht**-NH-dU-PPP-KKK-pep-**RPTL** + 1M NaOH ⟶
4. NH2-dU-PPP-KKK-pep-**RPTL** + **anhydride** (of Sch
5. (COO^-)-dU-PPP-KKK-pep-**RPTL** + thrombin ⟶
6. (COO^-)-dU-PPP-KKK-pep-NH2 + SE-Dye ⟶
7. (COO^-)-dU-PPP-KKK-pep-NH-Dye

FIG. 6F

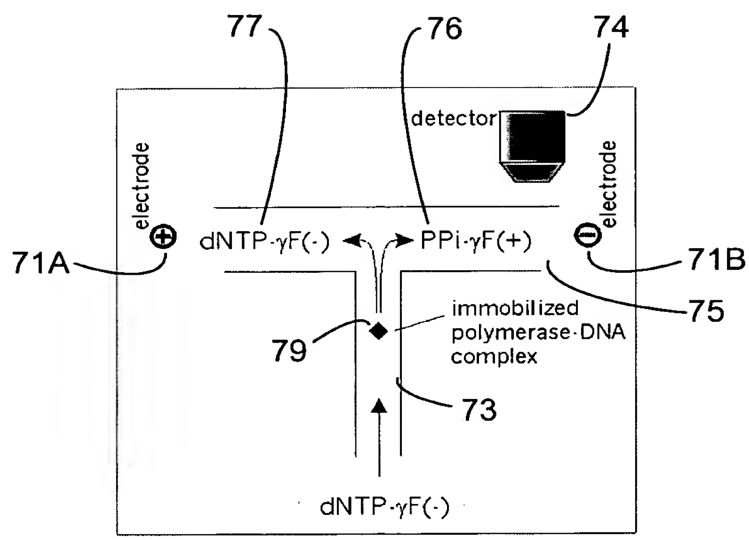

77
76
74
detector
electrode
electrode
71A
dNTP-γF(-)
PPi-γF(+)
71B
75
immobilized
polymerase-DNA
complex
79
73
dNTP-γF(-)

FIG. 7

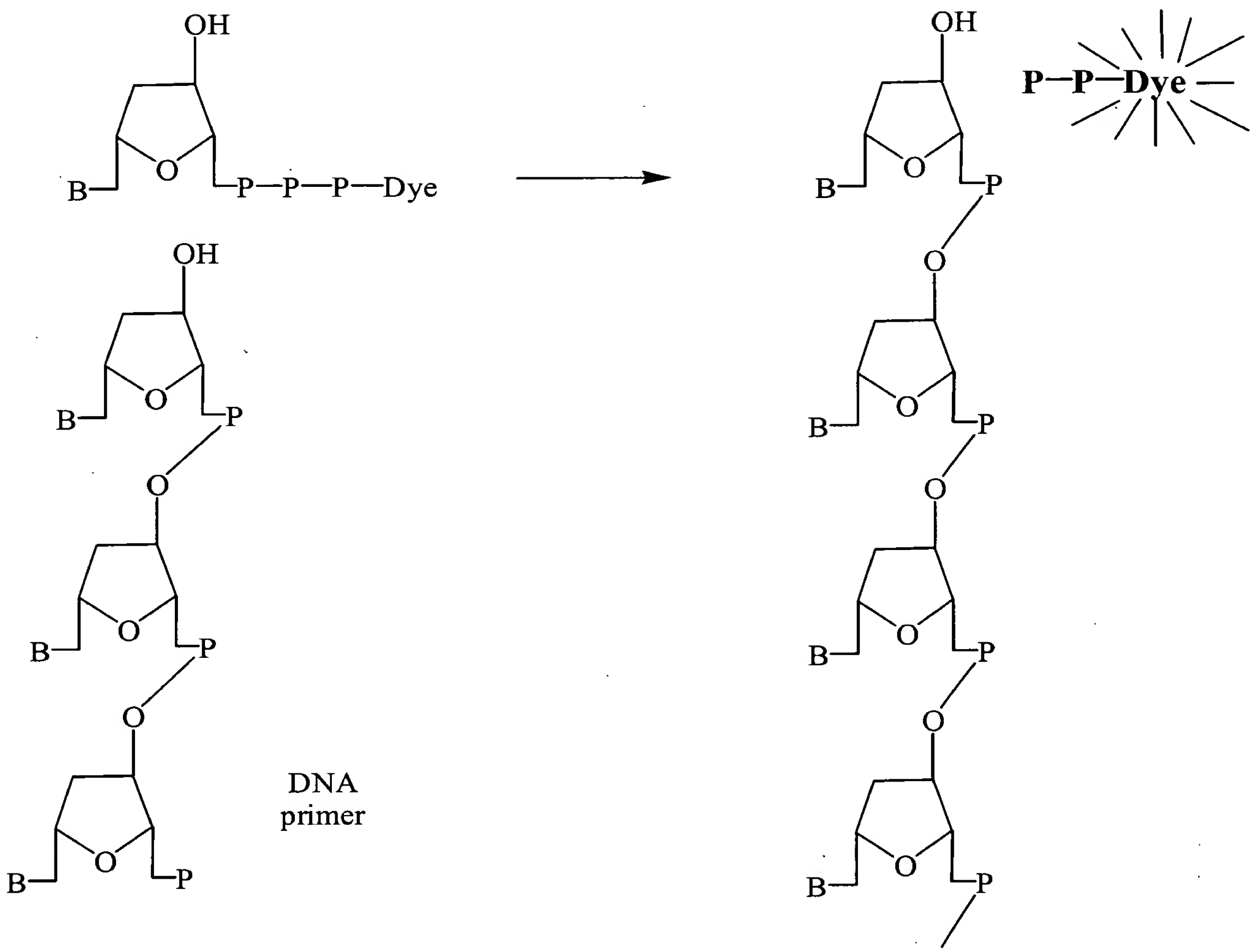

OH
B
O
P—P—P—Dye
OH
B
O
P
O
B
O
P
O
B
O
P
DNA
primer
OH
P—P—Dye
B
O
P
O
B
O
P
O
B
O
P
O
B
O
P

FIG. 8

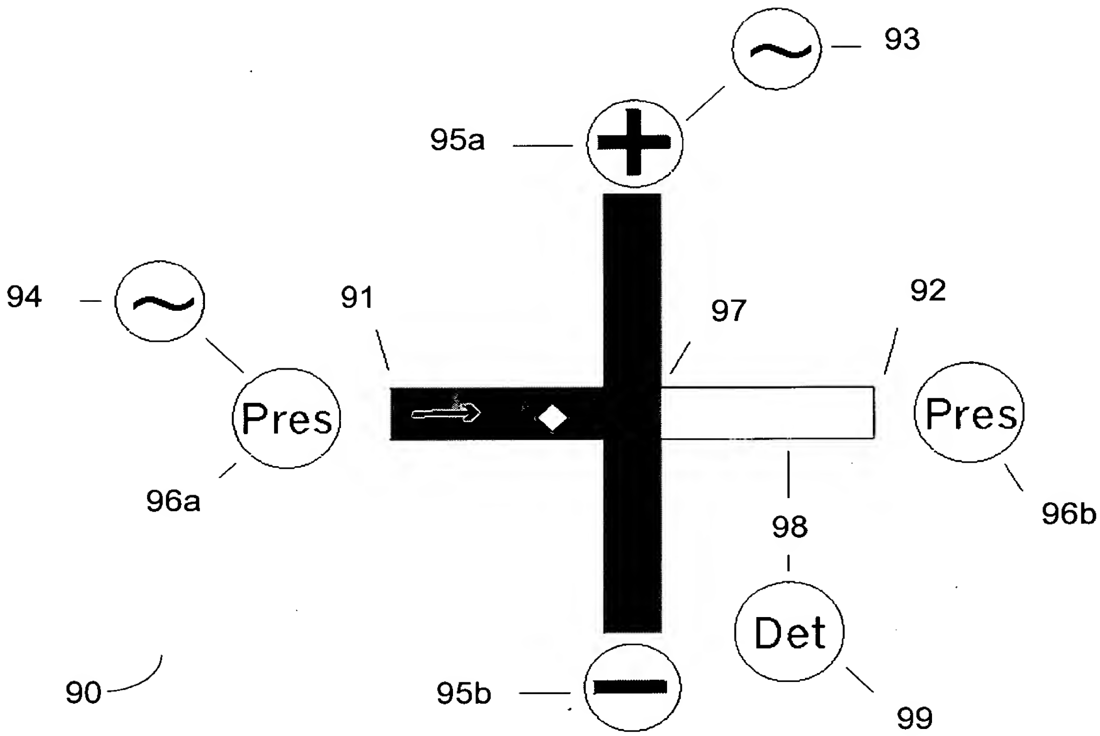


FIG. 9

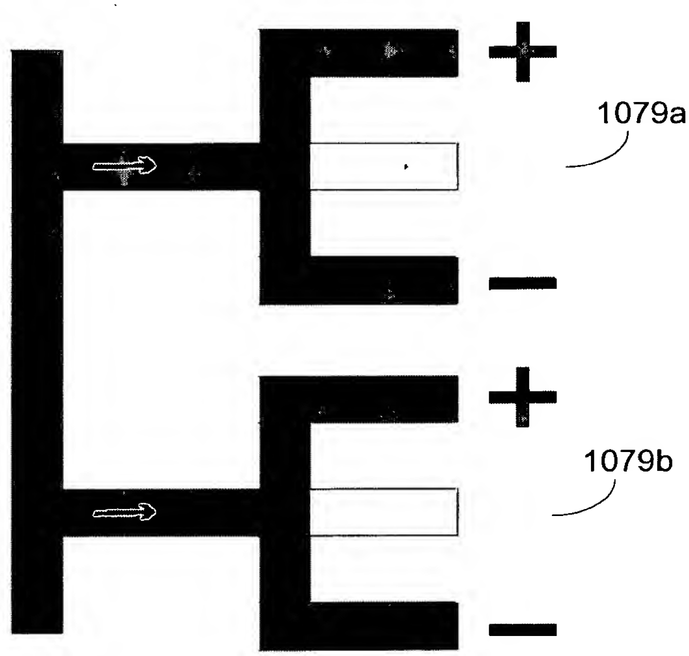
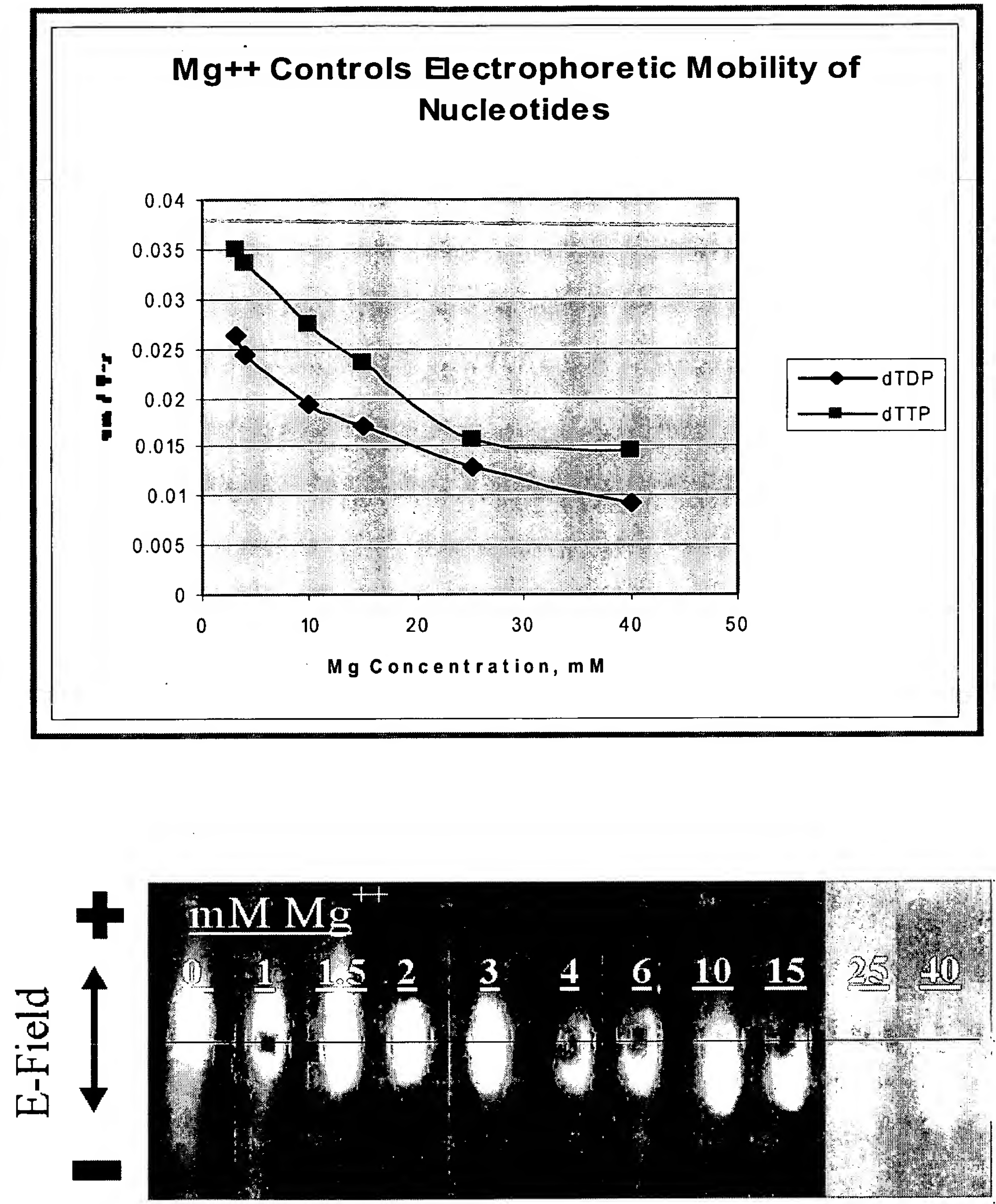


FIG. 10


Mg++ Controls Electrophoretic Mobility of Nucleotides
0.04
0.035
0.03
0.025
0.02
0.015
0.01
0.005
0
0
10
20
30
40
50
Mg Concentration, mM
dTDP
dTTP
mM Mg++
0
1
1.5
2
3
4
6
10
15
25
40
E-Field
+
-

A

B

FIG. 11

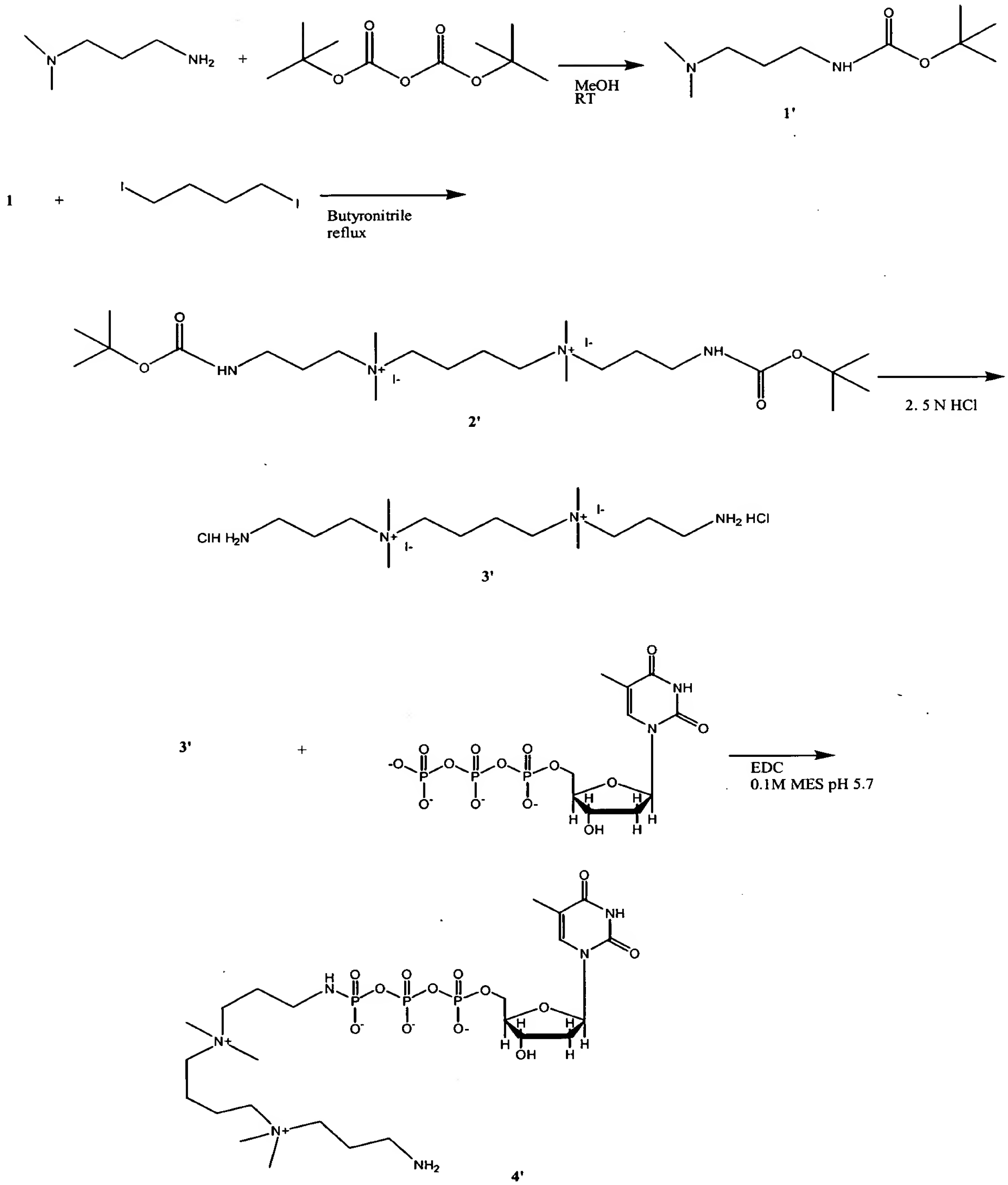

NH2
MeOH
RT
1'
1
Butyronitrile
reflux
2'
2. 5 N HCl
ClH H2N
NH2 HCl
3'
3'
EDC
0.1M MES pH 5.7
4'

FIG. 12

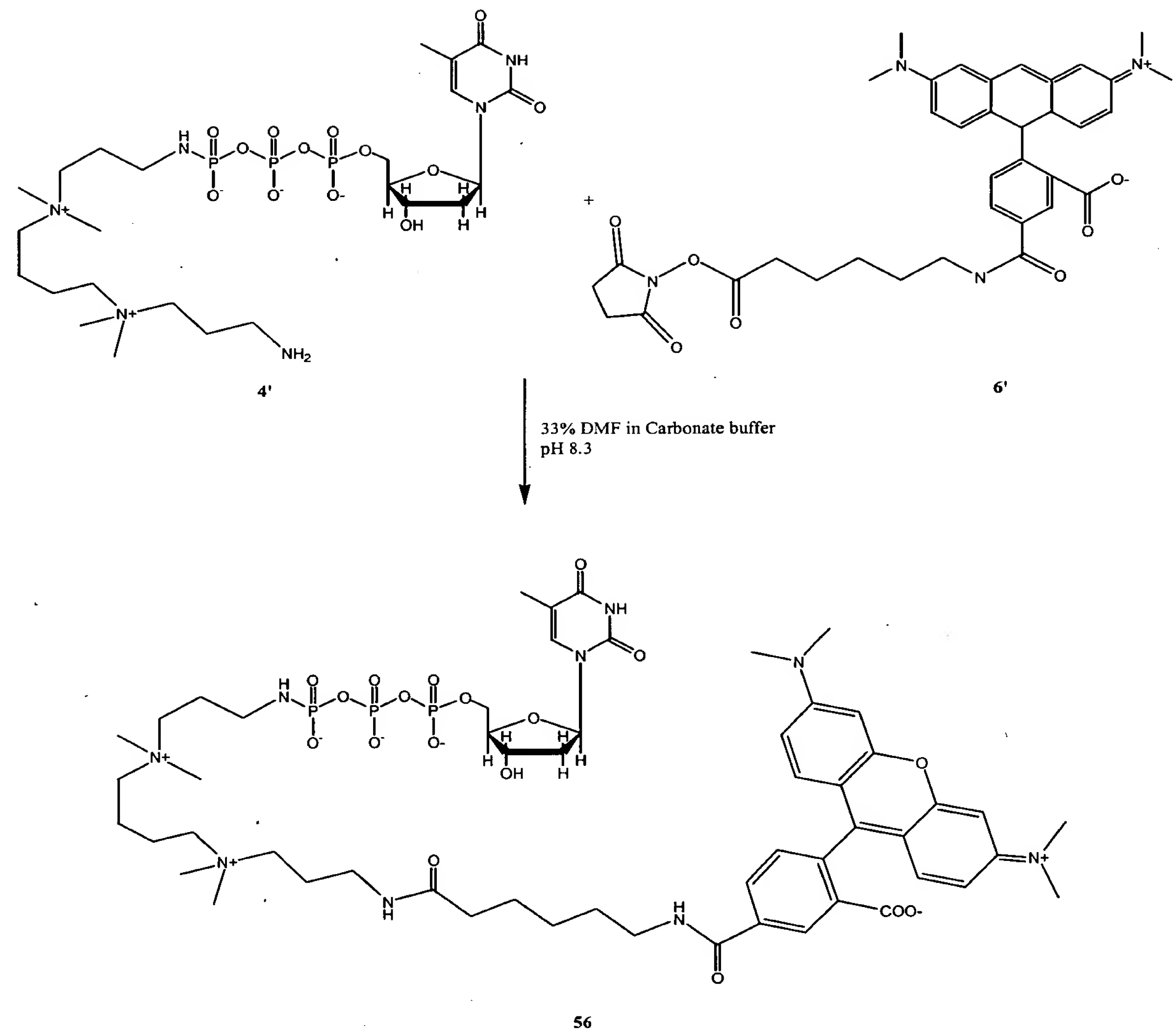


FIG. 12 (continued)